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NICOTIANA ARENTSII—A NEW, NATURALLY OCCURRING AMPHIDIPLOID, SPECIES

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In 1917, Winge pointed out that the arithmetic progression observed in the chromosome numbers of some genera might be the product of interspecific hybridization followed by chromosome doubling. Eight years later, Clausen and Goodspeed showed that such doubling had occurred in a fertile F_1 hybrid between *Nicotiana glutinosa* and *N. Tabacum*. This initial verification of Winge's hypothesis served to direct interest toward the incidence, causes and artificial induction of amphidiploidy (cf. Goodspeed and Bradley, 1942). It also led to an evaluation of the applicability of Winge's hypothesis in picturing evolutionary relationships in the genus *Nicotiana*.

Fundamentally, *Nicotiana* is a New World genus and, with the exception of 5 species showing 9 or 10 pairs of chromosomes, all its North and South American species possess either 12 or 24 pairs. Since there is no evidence that any of the 24-paired species is autopolyploid, it has been assumed that they were amphidiploid in origin (Goodspeed, 1934). Proof of such origin would contribute materially to the systematic botany of the genus and to an interpretation of its species distribution in the two Americas. For two of the 24-paired species, such proof is at hand. Thus, *N. Tabacum* (n = 24) has been shown to be

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an amphidiploid product of hybridization between progenitors of the modern species *N. sylvestris* (n = 12) and *N. tomentosa* (n = 12) or its relatives (Goodspeed and Clausen, 1928). These two species are peculiar to the Andes of Peru, Bolivia, or Argentina, and one of the products of the three University of California Botanical Garden Expeditions to the Andes has been the demonstration that today the distribution of the putative parental species overlaps. Similarly, progenitors of two other present day Peruvian-Bolivian-Argentine species, *N. paniculata* (n=12) and *N. undulata* (n=12), were undoubtedly the parents of the amphidiploid species *N. rustica*, the other well-known 24-paired species of commerce (Goodspeed, 1934, 1936).

Despite cytogenetic evidence in favor of amphidiploid origin, it has not been possible to reproduce artificially a duplicate of modern *N. Tabacum*, or of *N. rustica*, nor is there evidence of the existence of at least the former species in a wild state. In other words, although there is good reason to believe that the 24-paired American species of *Nicotiana* are of amphidiploid origin and while amphidiploidy has been induced in at least 27 experimentally produced F_1 interspecific hybrids (Bradley and Goodspeed, 1943), no naturally occurring American species has hitherto been found for which such origin could be shown.[1] It now appears that the recently discovered *N. Arentsii* is such a species.

In November 1938 one of the members of the Second University of California Botanical Garden Expedition to the Andes, Dr. César Vargas C., Professor of Botany and Director of the Botanical Museum in the University of Cuzco, Peru, found *N. Arentsii* in southeastern Peru, north of Lake Titicaca, near the Bolivian border, in and about the village of Limbani. Limbani is situated in the Dept. Puno, Prov. Sandia, at an altitude of about 3500 m., in a narrow box canyon on the eastern watershed of the Cordillera. The region is remote, climatically inhospitable, and little known botanically. Nearby, at somewhat lower altitudes, is the beginning of the Amazonian rain forest. The following extract from Dr. Vargas' notes is included here, in part because of its general botanical interest. Below Limbani, "the river of the same name runs swiftly and precipitately, a turbulent and foam-flecked stream which springs from the thawing snows of the nearby Cordillera. The village mostly consists of hovels and cabins nearly all made of whitish granitic rock with roofs of straw. The only street is the tortuous highway, lined on both sides by the principal habitations while the rest are scattered here and there on contiguous slopes. On the walls of the canyon are a series of shelves or terraces, now in ruins. Nobody knows how many hundreds of years ago they were constructed through the laboriousness and perseverance of a people who disappeared long

[1] Kostoff (1939) has commented upon the origin of a 32-paired race of the Australian species *N. suaveolens* (n=16) or its close relatives which was first grown in the University of California Botanical Garden (Wheeler, 1935). For this race Kostoff has suggested the name *N. Eastii* (but does not formally describe it as a new species) and considers it an amphidiploid product of hybridization between *N. suaveolens* and *N. maritima*. We have grown more than one 32-paired race from Australia and their morphological distinctions appear to complicate the problem somewhat.

ago and for whom the soil and the plant constituted the most cherished gifts of nature. . . . However, what is most cheering . . . is the abundance of vegetation . . . herbaceous and shrub forms as far as my eyes reach; even the rocky cliffs offer a varied flora. Along the road there are found several species of the families *Melastomaceae, Ericaceae, Campanulaceae,* etc. . . . beautiful and graceful clusters of *Bomarea* hang among *Baccharis, Cantua,* and other bushes; under the rocks and on the demolished terraces grow two species of *Begonia, Solanum, Centropogon, Monnina* and, in the dank turf, species of *Viola* and *Calceolaria.* The air was humid, the soil moist." The Quechua name for the new *Nicotiana* species was "Cjamasairi."

Four years later, Mr. Roy D. Metcalf, a member of the Third University of California Botanical Garden Expedition to the Andes, during an exploration of the Inambari and Tambopata drainage basins, collected *N. Arentsii* again. He found it restricted to the immediate vicinity of the village of Limbani and commonly around habitations, in some cases growing on walls and fences. According to his report, it prefers semi-shade, moist but not wet surroundings and gravelly soil among rocks. The preference of *N. Arentsii* for disturbed ground and the partial protection provided by habitations is characteristic of many South American species of *Nicotiana.* In Limbani the Indians did not make any use of the plant. Although Mr. Metcalf's itinerary northward from Chucuito (Dept. and Prov. Puno) at the northwest end of Lake Titicaca, took him as far as the Santo Domingo mine (immediately north of the Inambari river) in the province of Sandia and as far east as the towns of Sandia and Cuyocuyo, he did not again encounter this species. He states that *N. undulata* was also called Cjamasairi by the natives.

From the original seed of *N. Arentsii* collected by Dr. Vargas, 100 plants have been grown in the University of California Botanical Garden at Berkeley, California. Of these, 25 were grown in 1940, 75 in 1941. In the former year, all plants were set out in open ground; in the latter year, 35 were planted out, 38 held outdoors in large pots, and two allowed to flower in pots in the greenhouse. Potted plants from seed sown on January 10, 1941, began to bloom August 28, 1941. Those plants transplanted to open ground from seed sown on February 16, 1940, and January 10, 1941, began to bloom in the middle of the next October. To test their reported perennial capacities the field culture was not discarded at the close of the 1940 growing season. Although the winter (of 1940) was moderate in temperature, the plants died after setting seed. All plants held in large pots failed to develop lower branches. Their inflorescences were narrower than those of field plants. Finally, plants blooming in the greenhouse lacked the rose-red pigmentation in calyx and corolla, characteristic of field grown plants.

In all these garden cultures the branching was variable and frequently asymetric. There was also a strong tendency toward fasciation of elements of the inflorescence. Generally, the union did not extend beyond the first leaf of the axillary shoot. The branch thus affected would depart from the main axis

at a decidedly more acute angle than that of a normal shoot. Fasciation did not appear in a population of 13 plants grown, under equivalent conditions, in 1943 from selfed seed of a 1942 plant. Despite certain definite but intergrading variations in leaf shape and inflorescence type, the appearance of the original populations was as uniform as might be expected from seed of wild plants.

On the basis of herbarium and garden studies, *N. Arentsii*[2] was found clearly to combine prominent morphological features of two other species of *Nicotiana*—i.e., *N. wigandioides* Koch and Fint[2] and *N. undulata* R. and P.[2] The former is a subarborescent species, less massive than *N. tomentosa* but attaining a height of several meters and capable of living a number of years in the Berkeley climate. Especially on younger stem parts, long straight silvery hairs stand out prominently; there being, however, a glabrous region below the insertion of the leaf. The length, and to some degree the direction, of these soft, glistening hairs is modified in *N. Arentsii*. The red-purple coloration of *N. Arentsii* manifested in stem, leaf, calyx, and a pinkish blush to the corolla is derived from *N. wigandioides*. Plants of both species blossoming in the greenhouse instead of outdoors, lack it. Slow maturing, despite early sowing, is characteristic of both. *N. undulata* on the other hand, contributes to *N. Arentsii* relatively low stature and annual to limited perennial habit, greater leafiness in the inflorescence region, and a large adaxial calyx lobe. In certain plants it modifies the calyx coloration to a blue-black.

Generally speaking, *N. wigandioides* is a tropical or subtropical species as yet known only from central (western) Bolivia. It may be associated with the eastern headwaters of the Beni river for it has been collected a short distance beyond the edge of this drainage system (*Cárdenas* 2800, Inca Corral, Dept. Cochabamba, Prov. Chapare, 1700 m.; *Steinbach* 5754, Cerro de Incachaca, Dept. Cochabamba, Prov. Sacaba,[3] 2500 m.) and well within it in valleys in Dept. La Paz (*Rusby* 75a, Mulford Biol. Explor. Amazon Basin, Cañamina, Prov. Inquisivi, 1100 m.; *Troll* 2812, Lambate, Prov. Sud Yungas, 3400 m.; *Buchtien* 3905, Unduavi, Prov. Nor Yungas, 3200 m.; *Rusby* 2434, near Yungas, 1200 m.). It may also occur to the north and it is unfortunate that we have been unable to explore the tributaries of the Rio Beni in Bolivian territory adjacent to the Peruvian area where *N. Arentsii* was found.

Speaking of the vegetation at his last cited locality, H. H. Rusby (1888, p. 181) writes: "Descending to 7000 feet, we enter the great Andean forests which become heavier and heavier, though scarcely denser, as we descend. The trunks and greater branches are scarcely to be seen for the epiphytes upon them, chief of which are orchids, bromeliads, ferns, mosses, and aroids. At 5500 feet, we strike the coca and cinchona belt, and at 4000 feet we find the heat becoming oppressive and the air sultry. From 3500 to 5500 feet is prob-

[2] A detailed description of this new species appears at the end of this report where photographs of it and of *N. undulata* and *N. wigandioides* will be found.

[3] Undoubtedly a mistake and should read "the cánton of Sacaba, in Prov. Chapare."

ably the region of greatest rain-fall." Beyond general pictures such as this there is little description of habitat for *N. wigandioides* except the remarks of Dr. Martin Cárdenas on his collection of this species from Inca Corral. He refers to the region as "Ceja de Monte; the 'Ceja' is a kind of beginning of the Yungas." The term Yungas is used here as a collective designation for Bolivian tropical valley terrain. He states that *N. wigandioides* grows "on wet soil."

N. undulata is a widely distributed species which, by contrast, thrives in high, cold, bleak situations and generally fails to compete successfully as the climate becomes less rigorous and the region can support a richer vegetation. Apparently, it does not descend the eastern flank of the Andes but everywhere finds a congenial habitat in the Puna zone to the west. Its range is continuous from northern Argentina (Univ. Calif. Bot. Gard. Exped. Andes 6323, coll. *West*, 20 km. w. of Humahuaca, Prov. Jujuy, 3600 m.) to central Peru (Univ. Calif. Second Bot. Gard. Exped. Andes 10985, coll. *Stork*, Muilo, 10 km. w. of Tarma, Dept. Junin, 3300 m.) and it has even been found in northern Peru (Univ. Calif. Third Bot. Gard. Exped. Andes 30781, coll. *Metcalf*, road from Cajamarca, 3200 m.). Near Oroya, Peru, it forms almost pure colonies where only it and *Bidens* can tolerate the fumes from nearby smelters (Univ. Calif. Second Bot. Gard. Exped. Andes 10986, coll. *Stork*); between Candarave and Puno it occurs in the hard-packed roadbed (Univ. Calif. Third Bot. Gard. Exped. Andes 30387, coll. *Metcalf*); in Chucuito, Huancane, Putina, Asillo and other windswept altiplano towns of the Dept. Puno (*ibid.* 30677, 30704, 30718, 30424) it is common in the streets, on refuse dumps, and in the protection of houses and fences. Metcalf reports: "While crossing the altiplano, the one plant that one expects to find is this Nicotiana. When there is nothing else, there is always this . . . while never growing in great abundance in any one site, it none the less covers sparsely huge areas. Roughly, *N. undulata* occurs from 10,000 feet to over 14,000 feet."

Although *N. wigandioides* has not been found near the limited known range of distribution of *N. Arentsii*, the other species, *N. undulata*, which appears to have entered into its amphidiploid origin, occurs within 30 km. of it. In the La Paz region of Bolivia, *N. undulata* and *N. wigandioides* have been collected within this distance and in Dept. Cochabamba they are found within 10 km. of each other. It is, therefore, not too much to say that in past time these two species have been in contact, if they are not so at present. In the dissected eastern edge of the Peruvian-Bolivian Andes, alps and tropics are remarkably juxtaposed and wherever a cleared pathway opens, whether it be by diastrophic movement, streams, or man-made paths, *Nicotiana* species will seek to colonize the disturbed ground to the limit of their climatic tolerance.

Above, it has been pointed out that *N. Arentsii* is morphologically a mosaic of the distinctive characters peculiar to two other species of *Nicotiana*. Attention has also been called to the fact that, at one time, the range of distribution of these two species undoubtedly overlapped and that today one of them is known to be practically in contact with *N. Arentsii*. These two lines of evi-

dence suggest that this new species originated as a hybrid between progenitors of the two other species, chromosome doubling then occurring in the hybrid. The following cytogenetic observations largely confirm this suggestion.

It is, first, to be noted that the chromosome number of *N. Arentsii* is 24 pairs while both *N. undulata* and *N. wigandioides* possess 12 pairs. In an earlier report (Goodspeed 1934), it was shown that the somatic karyotype of *N. undulata* consists of chromosomes with median or submedian centromere, one of the submedian pairs satellited. In the case of *N. wigandioides* there are 8 pairs with median or submedian centromere and 4 with subterminal, one of the subterminal satellited. As regards size, there is little difference between the two complements; less, indeed, than between the largest and smallest chromosomes within a complement. Both karyotypes belong to the larger, but not the largest chromosome size groups in the genus. Detailed study of its chromosome morphology shows that the complement of *N. Arentsii* represents a summation of the two species types, with mostly median or submedian centromere but a few with subterminal. The scant size distinctions between the *N. undulata* and *N. wigandioides* complements do not, however, make it feasible to homologize full complements but it may be said that the morphological equivalents of the satellited pair characteristic of each of the two species has been identified in *N. Arentsii*.

Meiotic configurations (pollen mother cells) of each of the three species were studied to determine whether any characteristic *N. undulata* or *N. wigandioides* features reappear in *N. Arentsii*. In each of the 12-paired species, two IM bivalent types were selected as sufficiently distinctive to be identified: in *N. undulata* (1) a completely terminalized one-chiasma bivalent with oblong partners and (2) a completely terminalized thick ring bivalent; in *N. wigandioides* (1) an incompletely terminalized one-chiasma bivalent in which two droplet partners are separated by a central (generally small) bead and (2) a not quite terminalized, two-chiasma "diamond" bivalent. Each of these types reappeared in *N. Arentsii*.

In *N. undulata* and *N. wigandioides*, meiotic chromosome behavior is normal but in *N. Arentsii* pairing was sometimes found to be irregular, even in plants which produced only good pollen grains. It is not possible to say that this condition prevailed in every plant, as only a few were examined in first metaphase, but the fact that it was true of every plant examined is significant. It was noted that from 2 to 6 chromosomes (often in more than 50 per cent of the PMC) either failed to pair or formed trivalents. In 20 second metaphase counts of one plant, this irregularity reduced to 85 per cent the PMC showing 24–24 distribution of chromosomes, the remaining 15 per cent showing 23–25.

Beyond a determination of chromosome number, the cytology of *N. Arentsii* had not been studied at the time hybrids of this new species with *N. undulata* and *N. wigandioides* were made and grown. A few plants each of both F_1 hybrids were secured and inasmuch as the preponderance of meiotic material available proved to be from monosomic hybrids made with the same *N. Arentsii*

parent, it is clear that the particular *N. Arentsii* plant involved in the crosses was itself monosomic. Apart from somewhat complicating the determination of extent of pairing, this situation did not alter the essential evidence, briefly summarized as follows:

F_1 Hybrid	PMC counted	Plant number	Chromosome Constitution	Pairs per PMC Range	Pairs per PMC Average
N. undulata × *N. Arentsii*	14	F–341 P54	36	11–12	11.71
	25	F–385 P51	35	11–13	11.96
N. Arentsii × *N. wigandioides*	25	F–386 P55	36	11–13	11.92
	25	F–386 P58	35	11–13	12.08
	25	F–386 P59	35	11–13	11.96

In the case of F_1 *N. Arentsii* × *N. wigandioides*, somewhat more than 50 per cent of the counts represent 12 bivalents only. The remainder involve combinations ranging from $8_{II} + 3_{III}$ to 13_{II} with the largest class $10_{II} + 1_{IV}$ and the next $11_{II} + 1_{III}$. Almost the same types of association occurred in the case of F_1 *N. undulata* × *N. Arentsii* except that the proportion of 12_{II} was approximately 40 per cent. In view of the extent of multivalent formation in the two hybrids just mentioned, the occurrence of some pairing in the hybrid which represents the third side of the triangle, F_1 *N. undulata* × *N. wigandioides*, was not unexpected. Thus, a range of from 2 to 9 pairs was observed in 100 pollen mother cells while the average number of pairs was approximately 5.

For our present purpose, it is sufficient to call attention to the fact that the cytogenetic evidence shows that the gametic sets of both *N. undulata* and *N. wigandioides* are represented by a full set of homologous chromosomes in the gametic set of *N. Arentsii* and that the amount of pairing in F_1 *N. undulata* × *N. wigandioides* is low. In other words, the cytogenetic findings are consistent with the evidence from external morphology and from distribution which indicate that *N. Arentsii* represents an amphidiploid product of hybridization between those other two species of *Nicotiana*.

Nicotiana Arentsii Goodspeed, sp. nov.

Nicotiana herbacea; caule crasso, conspicue pubescente; foliis petiolatis, late ellipticis, rotundo-ovatis vel subcordatis; floribus in paniculas foliosas dispositis; calyce magno, lobis inaequalibus; corolla dilute straminea v. prope alba, obconico-clavata, limbo plus minusve zygomorpho, lobis obtusis; staminibus inclusis, abrupte curvatis, ad partem tubulatam corollae inferne affixis.

Type.—University of California Second Botanical Garden Expedition to the Andes, 1938–39, 9666 (U. C. Herbarium 665156). Coll. C. Vargas C., Nov. 21, 1938, **Limbani, Dept. Puno, Prov. Sandia, Peru.** Alt. 3400 m. Close to the road and houses. "Cjamasairi." *Other authentic material:* University of California Third Botanical Garden Expedition to the Andes, 1942–43, 30529. Coll. R. D. Metcalf, May 16, 1942, in town of Limbani, Dept. Puno, Prov. Sandia, Peru. Alongside huts, north end of town. Alt. 3350 m.

Slowly maturing annual or limited perennial 1–2 m. high. Stem commonly reddish purplish, rather densely clothed with distinct but not long, weak,

silvery-white hairs, ultimately oldest parts glabrate, thinly corky, woody within; main axis stiffly erect, 2–2 cm. thick, chief branches few, irregular in length and distribution, usually some long and rapidly ascending. Leaves pubescent; blade to 25 or 30 cm. long and about 20 cm. wide, rotund-ovate or subcordate or the uppermost often lance-ovate, base variable, apex acute or acuminate, veins evident, sometimes indenting upper surface a little, margin slightly undulate; petiole 3/8–1/10 as long as blade; frequently red coloration in petiole, midrib, veins, margin or even hairs. Inflorescence paniculate, 35–50 cm. long, narrow or broad, lower limits indistinct by very gradual reduction of leaves to sessile, broadly lanceolate bracts; central axis erect, thick, pubescent but seldom viscid; laterals numerous, frequently rather crowded, variable in length, generally rigid and spreading, paniculately or occasionally cymosely branched, ± viscid; ultimate pedicels about 2 mm. long, later 6 mm. Flowers abundant, odorless or nearly so. Calyx green, green suffused with red-purple, or green blended with blue-black, pubescent, 10–14 mm. long, broadly cylindric or subcampanulate; membranous area below each sinus narrow, short; lobes shorter than tube, broadly triangular-ovate, plane, unequal, largest never extending to corolla mouth. Corolla distinctly pubescent exteriorly except in the partially glabrous limb; tubular part 15–20 mm. long, 7–9 mm. wide, broadly obconic from short, narrower base, apically somewhat swollen immediately below the slightly contracted mouth, pale greenish yellow with pink or purplish tingeing above; entire limb (including rimmed, hexagonal mouth) to 17 mm. across, in bud spirally plicate and often bright pink, in flower spreading, pale straw color frequently conspicuously suffused with pink outside, rarely also some pink inside; lobes broad, obtuse, 3 upper ones smaller, commonly a little deflexed. Stamens inserted about 5 mm. from very base of corolla (at or near where the obconical expansion begins); filaments thick, pale greenish, 4 equal or subequal, wooly and erect for several mm. above insertion, then glabrous and curving outward abruptly to rest against corolla, extending to about 1 mm. below corolla mouth, 1 sigmoid, not wooly above insertion, commonly appearing somewhat shorter; anthers yellow-green. Pollen pale straw color. Hypogynous disk thick, red or red-orange. Ovary rotund-ovoid, greenish or purplish; style thick, faintly greenish; stigma green, discoid-pulvinate, median groove shallow. Capsule included, 7–9 mm. long, elliptic-ovoid, brown, on dehiscence upper half split into 2 divergent, notched valves. Seeds broadly elliptic-ovoid, 0.7–0.8 mm. long, rather dark brown, surface shallowly reticulate, meshes moderately large, deeply lobed by bounding ridges, embryo straight. Somatic chromosome number 48.

Distinguishable from *N. undulata* R. & P. by later maturity; longer, more distinct, glistening hairs; larger, rotund-ovate leaf; larger flower; longest calyx lobe plane, not boat-shaped in flower or fruit; corolla limb sometimes pinkish, never greenish; from *N. wigandioides* Koch & Fint. by shorter life span; smaller leaf; markedly unequal calyx segments; smaller corolla limb.

Named in honor of Mr. George Arents, to whom botany is indebted for a comprehensive compilation of the history and culture of tobacco.

SUMMARY

A new species of *Nicotiana, N. Arentsii* (n = 24), from southeastern Peru is described, with comments upon its morphology, distribution and cytology. Similar comments are made in the case of two other *Nicotiana* species, natives of Peru and Bolivia—*N. undulata* (n = 12) and *N. wigandioides* (n = 12). The morphological and distributional evidence points to an amphidiploid origin for *N. Arentsii* in which the other two species are involved. This hypothesis is confirmed by the cytology of the three species and of the F_1 hybrids made between them.

(Since the above was written, it has been possible to reëxamine the herbarium material of two Bolivian collections of H. H. Rusby which, before *Nicotiana Arentsii* was recognized, were identified as *N. wigandioides*. It is now clear that they were taken from plants of *N. Arentsii*. Both are Rusby's No. 822 and both are from Mapiri (Dept. La Paz, Prov. Larecaja) but one is labelled "5000 ft., April, 1886" and the other "2500 ft., May, 1886." Mapiri is approximately 200 km. southeast of the Limbani area in which the collections of *N. Arentsii* described above were obtained.)

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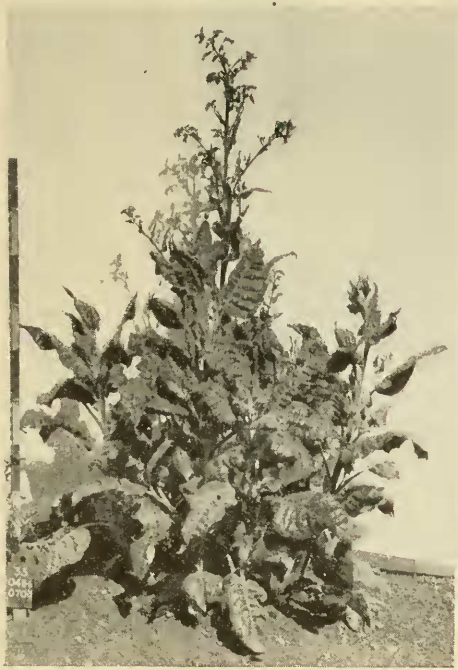
EXPLANATION OF PLATES

PLATE 26

Left: *Nicotiana Arentsii,* grown from seed of Univ. Calif. Second Bot. Gard. Exped. Andes 9666, Limbani, Dept. Puno, Peru, coll. C. Vargas C.

Center: *N. undulata,* grown from seed of Univ. Calif. Bot. Gard. Exped. Andes 7173, Pisacc, Dept. Cuzco, Peru, coll. James West.

Right: *N. wigandioides,* grown from seed collected by José Steinbach, near Cochabamba, Bolivia.



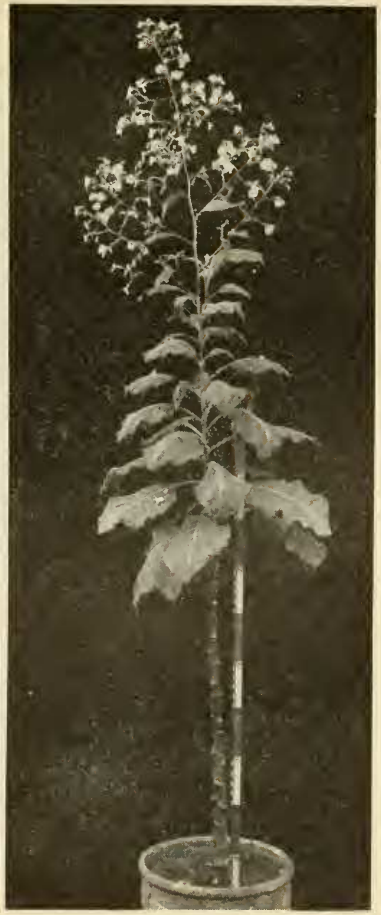


PLATE 27

Upper row: *Nicotiana undulata,* from seed collected by A. Weberbauer, at Huancavelica, Peru. The species is polymorphic in corolla shape and size. The race figured is relatively large flowered with the tubular part of its corolla broad and little dilated below the mouth.

Middle row: *N. Arentsii,* source as in plate 26.

Lower row: *N. wigandioides,* source as in plate 26.

(Black paper has been inserted in the corolla tubes of the sectioned flowers and one or two stamens removed to show the pistil. In *N. undulata* and *N. Arentsii* those removed included a short stamen; in *N. wigandioides* the two removed are no shorter than those remaining.)